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Lambda Integrase: Armed for Recombination

Bacteriophage λ moves its viral genome into and out of the bacterial chromosome using site-specific recombination. Crystal structures of reaction intermediates in this recombination pathway provide exciting new snapshots of full length λ integrase interacting with both core and regulatory DNA elements.

Gregory D. Van Duyne

Tyrosine recombinases are found in bacteria and yeast, where they catalyze a variety of important DNA transactions including integration and excision of bacteriophage DNA into and out of the host genome [1]. Structures of reaction intermediates from the Cre and Flp recombinase systems [2,3] have shed considerable light on the essential features of site-specific recombination in this family, but similar models for the more complex λ integrase system have until recently been elusive. The laboratories of Tom Ellenberger at Harvard Medical School and Arthur Landy at Brown have now taken this field to another level with crystal structures of reaction intermediates containing full-length λ integrase bound simultaneously to core and regulatory DNA elements [4].

To understand what is special about the λ integrase system, it is useful to start with Cre, which is perhaps the simplest tyrosine recombinase [5]. Cre has two protein domains that work together to bind to the *loxP* site, bring two *loxP* sites together and catalyze recombination. The Cre–*loxP* recombination assembly contains a tetramer of Cre subunits bound to two *loxP* sites (Figure 1). In contrast, λ integrase has an additional domain that binds to regulatory DNA sequences located in 'arms' that flank the core sites where strand exchange takes place [6,7]. As shown in Figure 1, the tetrameric assembly of four integrase subunits and two recombination sites is more complex in the λ integrase system, and it has been somewhat of a puzzle to conclude from biochemical data how all of the domains and binding sites are arranged. The arm binding domains in λ integrase play an absolutely crucial role in recombination; the catalytic and core binding domains cannot carry out 'Cre-like' recombination without them.

Two new crystal structures in the λ integrase system described by Biswas *et al*. [4] contain a Cre-like protein–DNA tetramer linked to a novel tetramer of arm binding domains that is in turn bound to two arm DNA sequences, as shown schematically in Figure 1. The first structure represents an intermediate where strand exchange has taken place between duplex substrates to form a four-way Holliday junction, but ligation has been blocked. The second complex was formed from an immobile Holliday junction substrate — that is, one whose branch point is defined and fixed by its sequence. The organization of catalytic and core binding domains on the four-way junction DNA are similar in most respects to that observed for the Cre and Flp systems [3,8]. The

real breakthrough in this work is also the most striking aspect of the new structures: the unexpected and intriguing organization of the arm binding domains and arm DNA sites relative to the rest of the complex.

The λ integrase arm binding domains form a tightly packed dimer of dimers that is connected by a short linker to the core binding (CB) domains. This tetramer of arm binding domains exposes two antiparallel surfaces that interact with the arm DNA sites. One might have guessed that the arm binding domains would interact primarily with their respective CB domains, resulting in a λ integrase subunit that has three domains stacked on top of one another. Instead, a cyclic domain swap occurs in the λ integrase tetramer, such that each arm binding domain sits primarily on an adjacent subunit's CB-domain (Figure 1).

The flexibility of the arm binding tetramer also appears to be different from that formed by the CB and catalytic domains. In the λ integrase, Cre and Flp systems, the CB and catalytic domains form a pseudo-four-fold symmetric tetramer on Holliday junction DNA substrates. The quaternary structure of this assembly is thought to convert between two distinct two-fold (but nearly four-fold) symmetric conformers during the transition between the first and second strand exchange steps of the reaction [8]. In contrast, the arm binding tetramer of λ integrase forms an elongated, 2-fold symmetric parallelogram of subunits that does not appear to be capable of interconverting between alternative forms while the arm sites are engaged (Figure 2).

With their experimental structures for the λ integrase tetramer bound to arm and core DNAs as a guide, Biswas *et al*. [4] generated models for the Holliday intermediates expected during the integration and excision pathways of λ integrase recombination. The crystal structure of integration host factor (IHF) bound to its

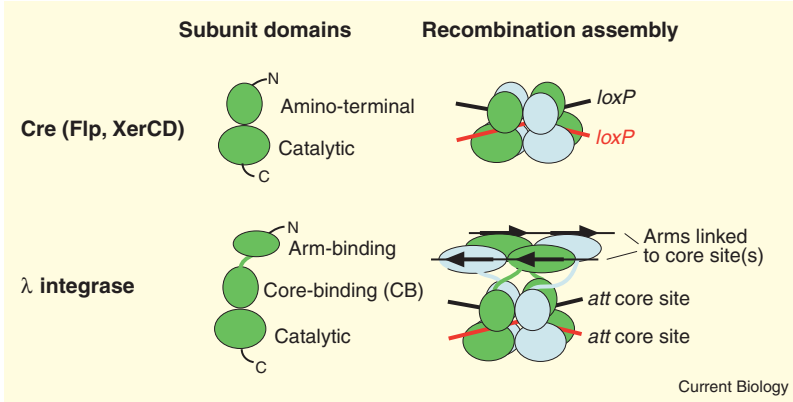

Figure 1. λ integrase compared to the simpler recombinases.

Tyrosine recombinases such as Cre have two domains that bind the core recombination sites and carry out recombination on their own. λ integrase has a third, amino-terminal 'arm binding' domain that binds to the arm region of the attachment site. The DNA complex cartoon for λ integrase (lower right) represents the new crystal structures reported by Biswas *et al*. [4].

recognition site [9] was used to model the sharp bends introduced in the arms at positions where IHF is known to bind. These models are shown in cartoon form in Figure 2. In both the integration and excision reactions, the P' arm simply forms a loop (with the help of IHF) between the core and arm binding sites and contacts one of the two available binding surfaces of the arm binding tetramer. The P arm contains multiple bends, crossing under the λ-int tetramer in the integration model and forming a large loop that changes direction in the excision model. The excision architecture in the Biswas *et al*. [4] model differs somewhat from that proposed earlier based on biochemical data [10], but it is easy to see why such predictions would be difficult, given the cyclic domain-swapped nature of the arm binding domains. It also seems possible that the λ integrase tetramer could be rotated 90° with respect to the DNA substrates in one of the two reaction pathways, exposing orthogonal arm binding surfaces that would require re-routing of the arms in the corresponding model.

In addition to providing a model-building framework to help understand λ integrase recombination, the new structures raise a number of interesting possibilities regarding regulation of recombination, particularly with respect to the arm binding tetramer. The arm binding domains, along with arm sequences and auxiliary DNA-bending proteins, provide λ integrase with a level of regulation that is not possible in the simpler tyrosine recombinases (for example, *attL* and *attR* do not just recombine to give back *attB* and *attP* following integration). It has been known for some time that the arm binding domains of λ integrase provide high affinity interactions with *att* site DNA and therefore contribute substantially to the 'glue' that holds the recombination assembly together. More recently, however, it has been shown that the interaction with arm DNA serves more than just an architectural role and that the arms play an active role in the recombination reaction [11].

Biswas *et al*. [4] suggest in their analysis of the λ integrase–DNA structures that the arm binding tetramer may influence the recombination reaction by imposing restraints on the quaternary structure of the complex. This could be accomplished if the arm binding tetramer favors the quaternary structure associated with the second half of the reaction, where the second pair of strands are exchanged (Figure 2). In this allosteric model, the equilibrium

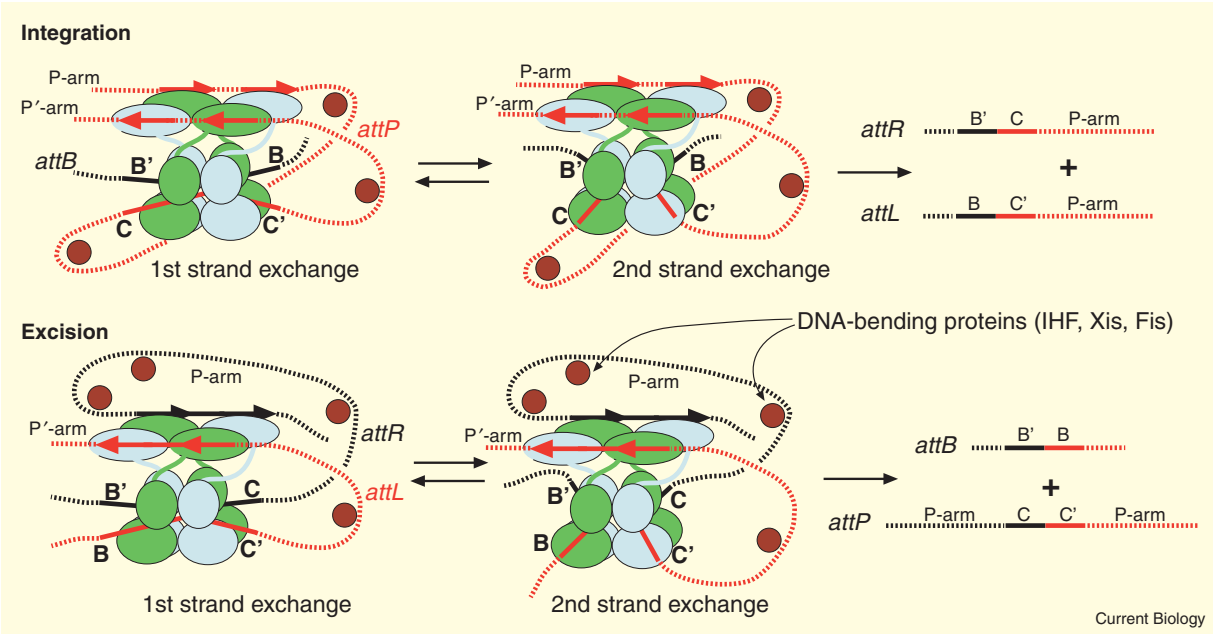

Figure 2. Integration and excision by λ integrase.

Schematic representation of the integrative and excisive pathways, based on the models proposed by Biswas *et al*. [4]. The first and second strand exchange cartoons represent the first and second halves of the recombination reaction, respectively. In the first half of integration, for example, λ integrase brings *attP* and *attB* sites together and exchanges the first pair of strands to generate a Holliday junction intermediate. In the second half of the reaction, the Holliday intermediate has isomerized to form a distinct quaternary structure and exchange of the second pair of strands generates recombinant *attL* and *attR* products.

between synapsed substrates and recombinant products is shifted towards product formation, so that the reaction 'runs downhill' once it gets started.

An additional component in allosteric regulation of recombination could involve the accessory DNA-bending proteins such as integration host factor (IHF), which play an important role in establishing the architecture of the recombination assembly by forming tight turns in the arm DNA. One might imagine that the conformational energy stored in these bends, along with supercoiling of the substrate, could be used to promote certain aspects of recombination as was suggested for Tn10 transposition [12]. In any case, it is clear that three-dimensional models of the intact λ integrase recombination system will provide fertile ground for generating and testing ideas about how this complicated machine functions.

In summary, decades of biochemical and genetic data in the λ-int recombination system have been patiently waiting to be complemented by three-dimensional models of a reaction in progress. With the remarkable structures described by Biswas *et al*. [4], that day has finally come and the lambda family album has some fantastic new snapshots [13].

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